## Phylogeny

PAN3 orthologs are conserved across eukaryotes, including *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *Neurospora crassa* (Nc), *Saccharomyces cerevisiae* (Sc), *Chaetomium thermophilum*, *Xenopus*, mouse, and *Caenorhabditis elegans* (verma2024pandeadenylaseensures pages 1-5, unknownauthors2014structuralcharacterizationof pages 110-111, wolf2014structuralbasisfor pages 4-5, unknownauthors1980functionsofdeadenylation pages 70-80, brown1996pan3encodesa pages 3-4, christie2013structureofthe pages 4-5). Although PAN3 contains a domain with a kinase-like fold, it lacks conserved catalytic residues required for phosphotransferase activity and is therefore classified as a pseudokinase (verma2024pandeadenylaseensures pages 1-5, christie2013structureofthe pages 4-5, unknownauthors2014structuralcharacterizationof pages 108-110). Consequently, PAN3 is not included as an active kinase in the kinome classifications by Manning et al. (verma2024pandeadenylaseensures pages 1-5, unknownauthors1980functionsofdeadenylation pages 55-59, wolf2014structuralbasisfor pages 2-3, christie2013structureofthe pages 4-5, brown1996pan3encodesa pages 6-7).

## Reaction Catalyzed

PAN3 is the non-catalytic, regulatory subunit of the PAN deadenylase complex (martin2014panoramathreeconvergent pages 1-2, brown1996pan3encodesa pages 7-9). The PAN2-PAN3 complex catalyzes the 3’-to-5’ exonucleolytic hydrolysis of the polyadenylate [poly(A)] tail of an mRNA substrate (schafer2019molecularbasisfor pages 1-3, yan2014deadenylationenzymesregulation pages 4-5). This reaction involves the hydrolytic cleavage of phosphodiester bonds, which progressively removes terminal adenosine residues from the 3’ end of the RNA (zhang2023thedynamicpoly(a) pages 2-4, unknownauthors2020structuralandbiochemical pages 114-119). The products of the reaction are a shortened RNA molecule and adenosine monophosphate (AMP) (martin2014panoramathreeconvergent pages 1-2, schafer2019molecularbasisfor pages 1-3, wolf2014structuralbasisfor pages 8-9).

## Cofactor Requirements

The catalytic activity of the PAN2-PAN3 deadenylase complex is dependent on metal ions, typically Mg²⁺ (unknownauthors2020structuralandbiochemical pages 30-35, unknownauthors2020structuralandbiochemical pages 77-80, unknownauthors2020structuralandbiochemical pages 35-39). The pseudokinase domain of PAN3 binds ATP (unknownauthors2014structuralcharacterizationof pages 108-110, wolf2014structuralbasisfor pages 4-5).

## Substrate Specificity

As a pseudokinase, PAN3 lacks kinase substrate specificity (verma2024pandeadenylaseensures pages 1-5). The PAN2-PAN3 complex specifically targets the 3’ poly(A) tail of mRNA substrates (wolf2014structuralbasisfor pages 8-9, unknownauthors2020structuralandbiochemical pages 121-124, tang2019theintrinsicstructure pages 8-10). Substrate specificity is enhanced by the interaction between PAN3 and the Poly(A)-Binding Protein (PABP), which binds to poly(A) tails and recruits the PAN2-PAN3 complex to the mRNA (mangus2004positiveandnegative pages 10-12, wolf2014structuralbasisfor pages 2-3, unknownauthors2020structuralandbiochemical pages 170-174, unknownauthors2020structuralandbiochemical pages 127-131). The complex recognizes the intrinsic stacked, helical structure of poly(A) RNA; the presence of guanosine residues within the poly(A) tail disrupts this structure and strongly inhibits deadenylation activity (tang2019theintrinsicstructure pages 8-10, unknownauthors2020structuralandbiochemical pages 77-80, unknownauthors2020structuralandbiochemical pages 185-189).

## Structure

PAN3 consists of an N-terminal region, a central pseudokinase (PK) domain, a coiled-coil (CC) domain, and a C-terminal knob (CK) domain (unknownauthors2014structuralcharacterizationof pages 110-111, wolf2014structuralbasisfor pages 2-3, unknownauthors1980functionsofdeadenylation pages 64-70). The N-terminal domain is intrinsically disordered and contains a CCCH-type zinc finger that binds poly(A) RNA and a PABP-interacting motif 2 (PAM2) (unknownauthors1980functionsofdeadenylation pages 70-80, unknownauthors2020structuralandbiochemical pages 35-39, verma2024pandeadenylaseensures pages 1-5). The PK domain adopts a typical kinase bilobal fold but lacks key catalytic residues (christie2013structureofthe pages 4-5). The central CC domain mediates the formation of an intertwined, asymmetric homodimer, which is essential for function (unknownauthors2014structuralcharacterizationof pages 110-111, christie2013structureofthe pages 11-12). The C-terminal domain is critical for interaction with the PAN2 subunit (unknownauthors1980functionsofdeadenylation pages 64-70). The PAN3 homodimer assembles with a single PAN2 subunit to form a heterotrimeric complex (zhang2023thedynamicpoly(a) pages 2-4, wolf2014structuralbasisfor pages 8-9). PAN3 dimerization also creates a tryptophan-binding pocket that mediates interaction with GW182 proteins (unknownauthors2014structuralcharacterizationof pages 108-110).

## Regulation

PAN3 is regulated by post-translational phosphorylation (verma2024pandeadenylaseensures pages 1-5, unknownauthors1980functionsofdeadenylation pages 55-59). In yeast, Pan3p is phosphorylated by the Pho85-Pcl1 cyclin-dependent kinase at residues T57 and S252 (unknownauthors1980functionsofdeadenylation pages 55-59). Mammalian PAN3 is a substrate of Cdk5 (verma2024pandeadenylaseensures pages 1-5). Phosphorylation status modulates PAN3’s interaction with PABP and influences its subcellular localization (verma2024pandeadenylaseensures pages 1-5, unknownauthors1980functionsofdeadenylation pages 70-80). In NIH 3T3 cells, hypo-phosphorylated mutants cause the formation of large cytoplasmic P-bodies, while hyper-phosphorylation mimics result in nuclear localization (unknownauthors1980functionsofdeadenylation pages 70-80). Nucleotide binding to the pseudokinase domain is also a regulatory mechanism that affects the stability and function of the complex (schafer2014thestructureof pages 1-11, christie2013structureofthe pages 11-12).

## Function

PAN3 is a regulatory subunit of the PAN deadenylase complex, localizing to the cytoplasm and mRNA processing bodies (P bodies) (unknownauthors1980functionsofdeadenylation pages 50-55, unknownauthors1980functionsofdeadenylation pages 55-59). It physically interacts with the catalytic subunit PAN2 and is required for its deadenylation activity (brown1996pan3encodesa pages 7-9). PAN3 recruits the complex to its substrates via multiple interactions: its PAM2 motif binds to PABP on polyadenylated mRNA, and its zinc finger domain binds poly(A) RNA (wolf2014structuralbasisfor pages 8-9, unknownauthors2020structuralandbiochemical pages 35-39). PAN3 also interacts with GW182/TNRC6 proteins, which recruits the PAN complex to miRNA targets and links its activity to miRNA-mediated gene silencing (unknownauthors2014structuralcharacterizationof pages 108-110, unknownauthors2020structuralandbiochemical pages 185-189). Other interacting partners include the yeast Dun1 kinase and the RNA-binding protein MEX3 (wolf2014structuralbasisfor pages 2-3, unknownauthors2020structuralandbiochemical pages 189-193). The PAN3-containing complex is essential for the initial shortening of long mRNA poly(A) tails, proper P body formation, and maintaining spindle integrity and cell survival during mitotic stress (unknownauthors1980functionsofdeadenylation pages 50-55, verma2024pandeadenylaseensures pages 1-5, verma2024pandeadenylaseensures pages 9-12).

## Other Comments

No disease associations for PAN3 mutations are reported in the provided literature (brown1996pan3encodesa pages 3-3, mangus2004positiveandnegative pages 1-2, christie2013structureofthe pages 11-12, verma2024pandeadenylaseensures pages 1-5). In *Saccharomyces cerevisiae*, deletion of *PAN3* abolishes PAN enzymatic activity and causes mRNAs to have longer poly(A) tails, though the cells remain viable (brown1996pan3encodesa pages 7-9, brown1996pan3encodesa pages 3-4). Yeast *pan3Δ* mutants exhibit sensitivity to the microtubule-depolymerizing drug nocodazole (verma2024pandeadenylaseensures pages 9-12). Mutations in the ATP-binding pocket of the human PAN3 pseudokinase domain impair mRNA degradation, while mutations that prevent PAN2 binding abolish the deadenylation function of the complex entirely (christie2013structureofthe pages 11-12).

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